THE SPERMATOGENESIS OF A DAPHNID, SIMOCEPHALUS VETULUS.

A Preliminary Paper.

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In Daphnids several generations of parthenogenetic females are followed by a sexual generation in which males are produced together with eggs requiring fertilization in order to develop. These eggs, when fertilized, hatch invariably into females which serve as stem mothers for a fresh series of parthenogenetic generations.

A study of the spermatogenesis of a Daphnid would therefore be of special interest in deciding the question regarding the specificity of spermatozoa in the determination of sex.

During the summer of 1912 males of *Simocephalus vetulus* were obtained in abundance from the fresh-water pools in the vicinity of the Marine Biological Laboratory at Woods Hole, Mass.

The liquid of Petrunkewitsch and Flemming's fluid (strong) proved to be the best fixing agents for the purpose.

The sections were stained with Heidenhain's hæmatoxylin and also with safranin and light green.

The immature testis is an elongate body consisting of a solid mass of spermatogonial cells with ill-defined boundaries and enclosed in a thin membranous wall which is carried out posteriorly as the vas deferens.

The spermatogonial cells are fairly uniform in size. Their nuclei are large and contain one or two nucleoli (Fig. 3, *a*).

A constant feature is the presence, here and there throughout the testis, of cells distinguished by their disproportionately large nuclei and nucleoli. They occur in all sizes varying from those of ordinary spermatogonia to giant forms shown in Fig. 1.

In more mature testes numerous regions or islands are discernible especially in the center in which cells may be found in

various stages of maturation, the cells of an island being in about the same stage.

As the cells in these islands advance in development a ring of undifferentiated cytoplasm remains about each island. This cytoplasm is continuous with that surrounding other islands and also with the cytoplasm of the giant cells (Fig. 2), which lie between the islands.

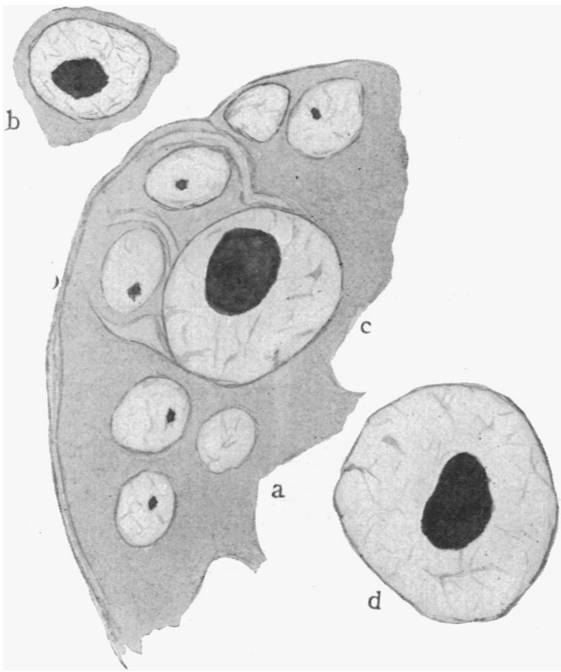

FIG. 1.

The islands remain distinct until their contents are transformed into free spermatids. The cytoplasm between the islands then disintegrates, producing large spaces in which lie the free spermatids. The testis thus acquires a lumen which ever increases in size until the testis becomes a hollow sac full of ripe sperm.

In the last stages, when the testis is a hollow sac whose walls consist merely of an epithelial coat, the giant nuclei are not to be found. However, there may be seen, scattered among the free sperm, irregular particles of disintegrating cytoplasm, possibly the remains of the rings of cytoplasm which formerly enclosed the islands.

The giant nuclei attain their greatest size in testes which are composed almost entirely of islands of spermatocytes in the various stages of maturation but in which no lumen yet exists.

Simultaneously with the growth of the giant cell the nucleus and noticeably the nucleolus increase in size. A change also takes place in the staining reaction of the nucleus. The chromatin network, which hitherto together with the nucleolus stained red with safranin, loses that capacity and takes up light green, an acid stain.

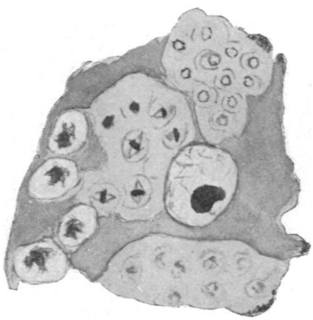

FIG. 2.

A well-grown giant cell thus possesses a large nucleus with an enormous basic staining nucleolus and an acid staining nuclear network, the granules in the surrounding cytoplasm staining red with safranin.

Similar cells have been described, in literature on spermatogenesis, as rudimentary ova. A significant fact, however, which militates against such an interpretation, at any rate for the giant cells in the *Simocephalus* testis, is that they grow directly from spermatogonia and do not pass through the synapsis stage.

The striking but superficial resemblance between these giant cells and growing oöcytes is evidently due to the one function common to both, viz., that of an enormous growth in size.

The ever-increasing size of the nucleolus during growth and its final dissolution in both types of cells favors the assumption that the nucleolus is intimately connected with cell growth.

In the spermatocytes in *Simocephalus* where no growth occurs the spermatogonial nucleolus remains small during synapsis and early disappears. The same is true for *Pandarus*[1] and for *Cyclops*.[2]

On the other hand, in spermatocytes where growth does occur, a growing nucleolus is described by Schmalz[3] in an Ostracod. In this form the nucleolus grows during synapsis and during the subsequent growth period to disappear on the formation of the spindle for the first maturation division.

[1] J. F. McClendon, *Arch. f. Zellforsch.*, V., 1909.

[2] R. Chambers, Jr., Univ. of Toronto Studies, Biol. Ser., No. 14, 1912.

[3] J. Schmalz, *Arch. f. Zellforsch.*, VIII., 1912.

The principal stages in the spermatocytic development of *Simocephalus* are indicated in Fig. 3. A resting spermatogonium is shown in Fig. 3, *a*. The spermatogonial chromosomes appear to be slender more or less U-shaped rods. During metaphase (Fig. 3, *b*), they are too closely massed to be counted. I have no doubt, however, that they are considerably more than eight in number. Nuclei in synizesis (Fig. 3, *c*) show a decided

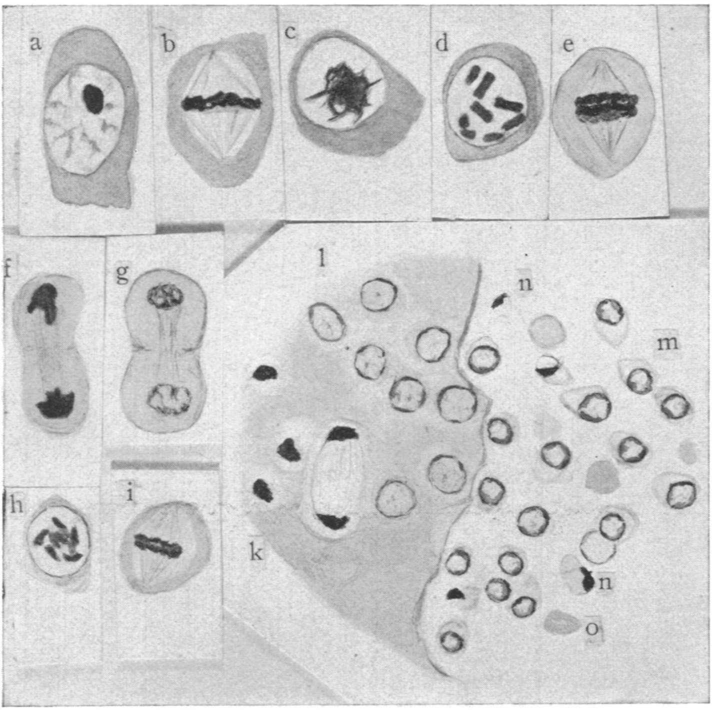

FIG. 3.

contraction of the chromatin threads. No growth occurs during this stage and no nucleolus is discernible. In the prophase of the primary spermatocyte (Fig. 3, *d*), eight distinctly double rod-like chromosomes are evident. The chromosomes in this stage are very distinct being more or less regularly distributed just under the nuclear membrane and in over fifty cases counted the double chromosomes were constantly eight in number.

Fig. 3, *e* and *f*, show the primary spermatocyte in metaphase and telophase. The chromosomes then pass into an interkinetic resting nucleus, Fig. 3, *g*. The resolution of the chromosomes of the two daughter cells into a resting nucleus is not always synchronous. One may often find an interkinetic nucleus at one end of the telophasic spindle while the chromosomes at the other end are still massed in a densely staining body. There is no doubt, however, that both ends pass into the resting state and form normal nuclei for one may find the entire contents of an island in interkinesis. And in still older islands all the cells pass into Metaphase II., leaving no cells behind. Fig. 3, *h*, represents the nucleus of a secondary spermatocyte in prophase. The chromosomes are approximately eight in number. They are shown in Fig. 3, *i*, in metaphase. Fig. 3, *k–o*, show the cellular elements lying in part of the lumen of a maturing testis and a portion of the adjacent wall. The secondary spermatocytes, Fig. 3, *k*, are shown in telophase. At *l* are spermatids with vesicular nuclei. Their arrangement in islands is better shown in the upper part of Fig. 2. Upon the disintegration of the surrounding cytoplasm, the spermatids come to lie in the lumen of the testis. Here, Fig. 3, *m*, the nuclei contract somewhat and become more densely chromatic.

In some of the spermatids, Fig. 3, *n*, *n*, the contents of the nucleus collects into a compact eccentric mass, which finally disintegrates and disappears.

In other spermatids the nucleus remains vesicular and it is this second type only that is to be found in the distal end of the vas deferens of a mature testis.

That approximately half of the spermatids degenerate is the impression gained by the examination of sagittal sections of entire testes.

Lepeschkin,[1] in a brief paper in Russian, kindly translated for me by Dr. M. Scholtz, of Cincinnati, on the spermatogenesis of the Daphnid, *Moina rectirostris*, describes occasional degenerating cells not only among spermatids but also among spermatocytes and spermatogonia. He speaks of the uniformity

[1] W. D. Lepeschkin, *Mem. Soc. Amis Sc. Nat. Anthrop. Ethnogr. Univ. Moscou*, Vol. 98, Sect. Zoöl., Vol. 3, No. 9, 1907.

of the cellular elements in young testes and of the formation of cysts enclosing spermatocytes, all the contents of a cyst being in the same stage of devêlopment. He does not describe nuclear changes, giving, as an excuse, the diminutive size of the cellular elements. His figures, showing degenerating cells, are not conclusive for one might easily confuse synizetic and interkinetic nuclei and possibly different stages of the giant cells with degenerative appearances. Occasional abnormalities do occur in any gonad but in healthy normal specimens studied by me I have been unable to discover degenerative appearances except among spermatids.

Two classes of spermatids, of which one only produces functional spermatozoa, are described by McClendon[1] in his studies in the spermatogenesis of *Pandarus sinuatus*, a parasitic Copepod, a species which exists only in the sexual state. According to McClendon some of the spermatids are transformed into "nutritive spheres." The "spheres" are a constant feature in *Pandarus* and the proportion formed is very large. On the assumption that spermatids occur in male- and female-producing classes, this condition might possibly disturb the sex ratio of the species. This is not true for I have collected large numbers and have always found the males and females in approximately equal numbers.

A personal study[2] of the spermatogenesis of *Pandarus sinuatus* has convinced me that McClendon's "nutritive spheres" are derived not from spermatids but from spermatocytes during interkinesis. If, then, we assume post reduction for the sex-determining factor, a condition which obtains in most forms where a distinct accessory or "sex" chromosome occurs, the formation of the "nutritive spheres" will be from neutral cells and should, therefore, cause no disturbance in the ratio of male- and female-producing sperm.

In Aphids[3] two classes of sperm are produced in the male differing in the presence and absence of an accessory chromosome. The class which does not possess the accessory chromo-

[1] J. F. McClendon, *Arch. f. Zellforsch.*, V., 1909.

[2] R. Chambers, Jr. In press.

[3] T. H. Morgan, *Proc. Soc. Exp. Biol. and Med.*, 5, '1908, and *Science*, Vol. 29, 1909. W. von Baehr, *Arch. f. Zellforsch.*, III., 1909.

some degenerates. By inference from other groups of insects this is the male-producing class. The other class produces functional sperm which, entering the egg, give rise to females only. These sperm correspond to the female-producing sperm of other insects.

In *Simocephalus* no accessory chromosome is to be distinguished, the sperm being apparently all alike in their chromosome number.

No means as yet have been found to distinguish two classes. The presence of degenerating sperm in the lumen of the testis does not necessarily prove the existence of two classes of sperm. However, as the functional sperm enter eggs which develop only into females, the assumption is permissible that these are the female-producing sperm and that the male-producing sperm are those which degenerate.